

Prevention of Herpes Keratoconjunctivitis in Rabbits By Silver Sulfadiazine

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Silver sulfadiazine, at a concentration of 10 $\mu\text{g/ml}$ when applied immediately after infection by *Herpesvirus hominis*, prevented the development of acute herpetic keratoconjunctivitis in rabbits. The efficacy was inversely related to the size of inoculum. Subclinical infections were observed in the drug-treated eyes.

Silver sulfadiazine, an antibacterial agent for the prevention and treatment of burn sepsis (1, 5, 6), has been shown to possess antifungal (7), antiherpesviral (2), and antitreponemal (3) activity in vitro. The present report describes its in vivo antiherpesviral activity in rabbits.

MATERIALS AND METHODS

Silver sulfadiazine was supplied by Marion Laboratories, Inc., Kansas City, Mo. (10,000 $\mu\text{g/ml}$ in a diluent containing 0.3% xanthan gum). The suspension was diluted in 0.85% sterile saline, to a concentration of 10 $\mu\text{g/ml}$, before use. Xanthan gum (0.3%), similarly diluted in saline, was used as a control.

Herpesvirus hominis type 1 (Rodanus strain) was used throughout the experiment. A pool, made in primary human amnion cell culture, contained $10^{6.5}$ mean tissue culture doses per 0.1 ml.

Viral inoculation was performed with an eye dropper. Two drops (0.1 ml) were instilled into the conjunctival sacs. The eyelids were closed manually, and gentle massage was applied for 10 s.

The drug was applied with an eyedropper. Two drops of silver sulfadiazine (10 $\mu\text{g/ml}$) or silver nitrate (0.1% solution) was applied to the conjunctival sac of the right eye. Xanthan gum (control), a similar diluted placebo, was instilled in the other eye. Treatment was given twice, once immediately, and the other 20 h after inoculation of the virus.

Cultures for virus were carried out 3, 5, and 7 days after infection. Material obtained from the conjunctiva of the lower eyelids was inoculated into primary cultures of human amnion cells maintained in minimal essential Eagle minimal essential medium containing 3% calf serum.

RESULTS

Prevention of keratoconjunctivitis. When treatment was started immediately after inoculation, complete protection was obtained after infection with 10^5 mean tissue culture viral doses; partial protection was observed when 10^6

mean tissue culture doses of virus was used (Table 1).

The effect of silver sulfadiazine on viral replication was less marked. Some of the infected eyes continued to excrete herpesvirus throughout the study period in the absence of objective evidence of keratoconjunctivitis. The quantity of virus excreted, however, was much smaller in the treated than in the untreated animals. This was indicated by the rapid rate and extent of development of the cytopathic effects in tissue cultures. Subclinical infections of the eye were not uncommon in the treated animals. Silver nitrate (0.1%), when applied immediately after infection and again 20 h later, failed to prevent infections (Table 2).

Clinical features. Treatment with silver sulfadiazine not only prevented the appearance of ocular infection but also death due to encephalitis (Table 3). The incubation period in animals that developed keratoconjunctivitis, especially in those given large inocula, was longer than in the controls. The duration of the disease was shorter, and the degree of inflammation was less intense after therapy. The untreated eyes showed edema and marked redness of both the conjunctivae and the cornea; the presence of mucopurulent exudate produced sticking of the eyelids. Photophobia was present. The treated eyes remained wide open; there was only slight redness of the conjunctivae and only minimal discharge.

DISCUSSION

A concentration of 10 μg of silver sulfadiazine per ml was selected for this study because it was found that, using this quantity of the drug, over 95% of 72 strains of herpesvirus were completely inactivated in vitro (6) and minimal ir-

TABLE 1. *Prophylactic effect of silver sulfadiazine on herpes keratoconjunctivitis in rabbits*

Inoculum (TCID ₅₀) ^a	Keratoconjunctivitis		Virus excretion	
	Treated	Control	Treated	Control
10 ⁶	1/4 ^b	4/4	2/4	4/4
10 ⁵	0/4	4/4	0/4	4/4
10 ⁴	0/4	3/4	0/4	3/4

^a TCID₅₀, Mean tissue culture infective dose.^b Number positive/number inoculated.TABLE 2. *Lack of prophylactic effect of silver nitrate (0.1%) on herpetic keratoconjunctivitis*

Inoculum (TCID ₅₀) ^a	Keratoconjunctivitis		Virus excretion	
	Treated	Control	Treated	Control
10 ⁶	3/3 ^b	3/3	3/3	3/3
10 ⁵	2/3	2/3	2/3	2/3

^a TCID₅₀, Mean tissue culture infective dose.^b Number positive/number infected.TABLE 3. *Effect of silver sulfadiazine on development of herpetic keratoconjunctivitis*

Eyes	Inoculum (TCID ₅₀) ^a	Infected/ total	Incubation period (days)	Duration of disease (days)	Deaths
Treated	10 ⁶	1/4	6	2	0/4
	10 ⁵	0/4			0/4
	10 ⁴	0/4			0/4
Untreated	10 ⁶	4/4	3	9	
	10 ⁵	4/4	5	8	
	10 ⁴	3/4	5	5	

^a TCID₅₀, Mean tissue culture infective dose.

ritation of the conjunctiva was produced. Although slight reddening of the bulbar and palpebral conjunctivae developed 4 to 5 h after instillation of the agent, this disappeared in 48

h. Higher concentrations produced more severe ocular manifestations.

The prevention of keratoconjunctivitis was probably due to direct inactivation of extracellular virus. Once viral absorption had taken place in tissue culture, the addition of silver sulfadiazine did not appear to prevent the development of the cytopathic effects. Many of the treated eyes continued to excrete herpesvirus, but in smaller quantities than the untreated ones. The reduction of viral infectivity probably also accounted for the prolonged incubation period, the shortened clinical course, and the mild nature of the lesions in the treated eye.

Silver nitrate did not prevent the development of keratoconjunctivitis. At higher concentration (1%), however, it was effective in alleviating the severity of corneal infection and reducing viral replication (4).

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